INNOVATIVE POTENTIALS IN CARDIAC SURGERY

Ph.D. Thesis

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LIST OF ABBREVIATIONS

Atrial septal defect	ASD
Congenital cardiac disease	CCD
Cardiolpulmonary bypass	CBP
Coronary bypass graft surgery	CABG
Dextrocardia	DX
Dilated cardiomyopathy	DCM
Double-outlet right ventricle	DORV
Extracorporal circulation	ECC
Enzyme-linked immunosorbent assay	ELISA
Fas ligand	FasL
Fluorescein isothiocyanate	FITC
Hypertrophic cardiomyopathy	HCM
Interleukin-6	IL-6
Left ventricular ejection fraction	LVEF
Left ventricular end diastolic diameter	LVEDD
New York Heart Association	NYHA
Off pump coronary artery bypass	OPCAB
Propidium iodide	PI
Pulmonary atresia	PA
Pulmonary stenosis	PS
Single ventricle	SV
Soluble form of Fas	sFas
Soluble form of the IL-6 receptor	sIL-6R
Tetralogy of Fallot	TOF
Transposition of the great arteries	TGA
Tumor necrosis factor-alfa	TNF-α

CONTENTS

1.1. ÖSSZEFOGLALÁS

A sebészi technika előrehaladása és a szívműködéssel kapcsolatos élettani-kórélettani ismeretek bővülése következtében a szívsebészet a szív- és érrendszeri betegségek kezelésének meghatározó tényezőjévé vált. A Szegedi Tudományegyetem Szívsebészeti Osztálya olyan oktatási, kutatási és gyógyítási egység, ahol minden összetett szívsebészeti beavatkozás elvégezhető. A betegellátás mellett, erőnkhöz mérten részt veszünk a klinikai kutatásban, új kórtani ismeretek megszerzésében és az elméleti tudás gyarapításában is. A szívsebészeti műtő változással teli környezet, amely elősegítheti az innovatív törekvéseket és erősítheti az együttműködést klinikusok és kutatók között. A dolgozat célkitűzése szerint tárgyaljuk és összegezzük tevékenységünk két fontos aspektusát: **(1)** a sebészi technika és technológia fejlesztésére irányuló törekvéseinket és **(2)** a kardiológiai alapkutatásokhoz csatlakozó kísérletes munkáinkat.

1. 2000. január 1. és 2005. december 31. között intézetünkben 5086 nyitott- illetve verő szíven végezett műtéti beavatkozás történt. Ezen időszak során a műszerezettségben és infrastruktúrában nem került sor jelentős fejlesztésekre, így folyamatosan szembesültünk azzal a ténnyel, hogy betegeink növekvő számának és az egyre bonyolultabb feladatot jelentő műtéti igényeknek csak akkor tudunk megfelelni, ha innovatív, a kihívásokhoz és a körülményekhez alkalmazkodó megoldásokat vezetünk be a mindennapi klinikai gyakorlatba.

Az extrakorporális perfúzió kedvezőtlen hatásainak kiküszöbölésére áttértünk a verő szíven történő koszorúér-áthidalás módszerére (OPCAB), amely mára fokozatosan, csaknem egyeduralkodóvá vált osztályunkon. A modern szívstabilizátorok és a szív megfelelő pozicionálásának segítségével teljes revaszkularizációt tudunk elérni. Az aorto-koronáriás shunt-ok alkalmazásával meg tudjuk előzni a szívizom iszkémiáját az anastomosis elkészítésének ideje alatt. Az intraaortikus ballonpumpa kiterjesztett használata ugyanakkor stabilizálja a keringést még akkor is, ha a szívet diszlokáljuk - a perioperatív szövődmények előfordulása így lényegesen csökkent.

2000 és 2005 között 1724 szívbillentyű műtétet végeztünk Szegeden. 2155 szájadékba 1781 mechanikus műbillentyűt, 376 xenograft billentyűt és 106 homograft billentyűt ültettünk be. Aorta billentyű csere pulmonalis autografttal (Ross-műtét) 32 esetben történt, emellett 400 valvuloplasztikát végeztünk.

1989-ben hozunk létre osztályunkon azt a laboratóriumot, amelyben homograftok preparálása, tartósítása és tárolása történik. 1990 óta homograftjainkat krioprezervációs technikával tartósítjuk és folyékony nitrogéngőzben tároljuk. Az utóbbi 6 esztendő során 106 esetben ültettünk be ezzel a módszerrel tartósított aorta billentyűt, 32 esetben végeztünk Ross-

műtétet, 12 pulmonalis billentyű csere, valamint 16 jobb kamrai kiáramlási pálya obstrukció miatti korrekciós műtét történt.

A Ross-műtét a mechanikus és xenograft billentyűk számos hátrányát kiküszöböli. 1999-óta foglalkozunk ezzel a technikával, felnőtteket és gyerekeket egyaránt operálunk, a 32 sikeres műtétből álló sorozat hazánkban a legnagyobb beteganyagot jelenti ebben a műtéti típusban.

2004-ben vezettük be osztályunkon a minimális behatolásból történő mitrális billentyű műtétek módszerét. Mindeddig 39 ilyen műtéti beavatkozásra került sor, halálozás vagy súlyos szövődmények nélkül. Jobb oldali axilláris minitorakotómiából végzett műtéteink kozmetikai eredményei kiválóak voltak, speciális technikával a lágyéki erek kanülálása is elkerülhető volt.

Hagyományos módszerrel nem operálható porcelán aorta esetén az aorta stenosis kezelésére megfordított, önkinyíló pulmonalis billentyűt használtunk. A billentyűt a bal kamra csúcsába helyeztük, és egy érprotézis közbeiktatásával az aorta ascendessel kötöttük össze. Az ilyen típusú műbillentyűnek ebben a formában történő alkalmazása első volt a világon.

Jobb kamrai kifolyó traktus obstrukció esetén, ha a kifolyótraktus transzannuláris folttal történő megnagyobbítást igényel, négy betegünknél monocuspidalis, egy billentyűtasakot tartalmazó pulmonális homograft foltot használtunk.

Korábban sikeresen alkalmaztunk homograft vena saphena magnát coronaria bypass műtéteink során. A kedvező tapasztalatok arra buzdítottak bennünket, hogy kiterjedten használjuk a homograft vénát a módosított Blalock-Taussig-műtétek során is. Eredményeink igazolták, hogy az emberi vena saphena allograft kitűnően alkalmas szisztémo-pulmonális shunt-nek is. Könnyen beszerezhető, beültetése egyszerű, csökkenti a vérzéses szövődmények kockázatát és a varratsor szivárgását is. Biológiai anyag lévén kevésbé kitett a fertőzéses szövődményeknek.

2. A klinikai munkát kiegészítő, multidiszciplináris együttműködés keretében végzett kísérletes vizsgálataink célkitűzése szerint adalékokat szolgáltattunk a szerzett szívbetegségek patomechanizmusának jobb megértéséhez. Többféle megközelítést alkalmaztunk azoknak az élettani és korélettani folyamatoknak megismeréséhez, amelyek szerepet játszhatnak a szívelégtelenség kialakulásában. E kollaborációs kutatási tevékenységeink közül kiemelendő azoknak a vizsgálatoknak a jelentősége, melyekben a tumor nekrózis faktor alfa (TNF-α) és az interleukin 6 (IL-6) citokinek szerepét hasonlítottunk össze a dilatatív és hipertrófiás kardiomiopátia etio-patogenezisében. Megvizsgáltuk a citokin választ, valamint a pro- és anti-

apoptotikus marker molekulák megjelenésének eltérő jellegzetességeit e két, nagy klinikai jelentőséggel bíró szívizom betegség esetén.

A vizsgálatok megállapításai szerint a dilatatív kardiomiopátiában szenvedő betegekben jelentősen megemelkedik a TNF-α, sFas, IL-6 és sIL-/R szintje, míg a hipertrófiás kardiomiopátiás esetekben csak magas IL-6 és sIL-6R szintek észlelhetők.

A szívizom minták Western blot analízisével a TNF-α, IL-6, Bcl-2 és Bax proteinek szintjét vizsgálva megállítottuk, hogy a dilatatív kardiomiopátiás betegekben a TNF-α, IL-6 és a Bax, míg hipertrófiás kardiomiopátiában csak a Bcl-2 expresszálódik fokozott mértékben. Adataink arra utalnak, hogy dilatatív kardiomiopátiás betegekben a pro-apoptotikus TNF-α és Fas útvonalak fokozott aktivációja dominál, míg hipertrófiás kardiomiopátiában ez az egyensúly az anti-apoptotikus irány felé tolódik el. E felismerés szerint az apoptózis és a citokin válasz eltérő szerepet játszik a kétféle kardiomiopátia patogenezisében.

1.2. SUMMARY

Advances in surgical techniques and knowledge relating to the cardiac function have made heart surgery the first-line procedure for cardiovascular diseases. As part of an academic medical center, and complementing the complex surgical techniques, the Department of Cardiac Surgery has participated in and acquired experience in clinical research as well. The purpose of this thesis is to discuss and summarize two main aspects of the above possibilities: **(1)** our efforts to improve surgical techniques and technologies (*"clinical core"*), and **(2)** serving as a start-up point for complementary basic research in cardiology (*"basic science core"*).

1. The operating room is a dynamic environment, facilitating the rapid exchange of ideas and innovations, and creates an optimal environment for collaboration. From January 01, 2000 to December 31, 2005, a total of 5086 open-heart or beating-heart coronary bypass operations were performed at our institution. With unchanging facilities, we were forced to find and introduce innovative methods to follow the expanding challenges and increasing requirements of our patients.

In an attempt to avoid the deleterious effect of the CBP, the *off-pump* technique (OPCAB) has been increasingly used, and has become the predominant method for coronary revascularization in our practice. With the use of advanced tissue stabilizers and heart-positioning methods, complete revascularization can be achieved. Application of an aorto-coronary shunt prevents myocardial ischemia during suturing of the anastomosis, and the extended use of intra-aortic balloon counter-pulsation prevents the hemodynamic instability when the heart is dislocated, and reduces the incidence of perioperative complications.

Between 2000 and 2005, 1724 patients underwent heart valve operations in Szeged, targeting 2155 orifices. 1781 mechanical valves, 376 xenograft valves and 106 homograft valves were implanted. We performed 32 aortic valve replacements using pulmonary valve autografts (Ross procedure) and 400 valvuloplasties.

A laboratory for harvesting, preserving and storing homografts was established in Szeged in 1989. Since 1990, homografts are cryopreserved and stored in liquid nitrogen vapor at -196 ^{o}C. During the last 6 years, these self-processed homograft valves were used for aortic valve replacement in 106 cases, and for the Ross procedure in 32 patients, while 12 pulmonary valve replacements and 16 corrections of a right ventricular outflow tract obstruction were performed.

The Ross procedure solved some of the inherent drawbacks of mechanical and biological (xenograft) valve substitutes. Since 1999, 32 patients have been operated on by this

technique at the Department, including both pediatric and adult cases, comprising the largest series of Ross procedures in Hungary.

Minimal access mitral valve surgery was introduced into clinical practice in Szeged in 2004, and 39 operations were performed up to the end of 2005, without any mortality or serious complications.

In cases of a porcelain aorta, we used a reversed injectable pulmonic heart valve. The operation was carried out by inserting the self-expanding valve into the apex of the left ventricle, and a vascular graft was used to connect the apex with the ascending aorta. This was the first application of this type of valvular prosthesis for this field of indication.

For right ventricular outflow tract reconstruction, when transannular augmentation of the obstruction is required, a pulmonary homograft patch containing one of the cusps of the pulmonary valve was implanted successfully in 4 infants so far.

We applied a novel method of right axillary minithoracotomy with excellent cosmesis. The operation can be performed without groin cannulation by using special venous and arterial cannulae through the incision.

Having employed some saphenous vein allografts successfully as coronary artery bypass grafts, we decided to use homograft veins for the creation of modified Blalock-Taussig shunts, first preferably in infected patients. Later, the preliminary good results encouraged us to use them more extensively. The results showed that human saphenous vein allograft is a good alternative biomaterial for the performance of a modified Blalock-Taussig shunt. It is easily available, its use simplifies the operative procedure, and it is devoid of bleeding complications and serous leakage. Being a biomaterial, presumably it is less prone to take part in infectious complications.

2. The aim of our experimental work was to provide a basis for collaborative, multidisciplinary investigations aimed at obtaining an insight into the pathomechanism of acquired heart diseases. Several routes were followed, searching for and studying physiological and pathophysiological pathways that may play a role in the evolution of heart failure. We compared the contributions of TNF-α and IL-6 to the pathogenesis of dilatative and hypertrophic cardiomyopathies, and explored a potential connection between the cytokine pattern and the pro-apoptotic or anti-apoptotic markers in these two different heart diseases.

Dilatative cardiomyopathy patients exhibited elevated concentrations of TNF-α, sFas, IL-6 and sIL-6R, while hypertrophic cardiomyopathy patients had only high IL-6 and sIL-6R levels as compared with healthy individuals. Western blot analysis of the levels of TNF-α, IL-6, Bcl-2 and Bax proteins in myocardium samples demonstrated that dilatative

cardiomyopathy patients express increased levels of TNF-α, IL-6 and Bax, whereas hypertrophic cardiomyopathy heart lysates display only elevated levels of Bcl-2.

We concluded that our data indicate a strong activation of the pro-apoptotic TNF-α and Fas pathways in dilatative cardiomyopathy patients, and an anti-apoptotic shift in hypertrophic cardiomyopathy patients. These findings have a bearing on the pathogenesis of the cardiomyopathies, since apoptosis may account for certain dysfunctions observed in dilatative while IL-6 may elicit the hypertrophy characteristic of hypertrophic cardiomyopathy.

2. LIST OF FULL PAPERS

1. Kovács GS, Fazakas S, **Bogáts G**, Rácz R. Measurement of resistance versus flow in assessing efficiency of aortocoronary bypass grafts. J. Cardiovasc. Surg. 1990; 31(5): 571-3. **IF: 0.80**

2. **Bogáts G**, Kertész E, Katona M, Tószegi A, Simonfalvi I, Kovács G. Modified Blalock-Taussig shunt using a saphenous vein allograft. Five-year experience. Orvosi Hetilap 1995; 136(24): 1263-6. **IF: 0**

3. **Bogáts G**, Kertész E, Katona M, Tószegi A, Kovács GS. Modified Blalock-Taussig shunt using allograft saphenous vein: six years' experience. Ann. Thorac. Surg. 1996; 61(1): 58-62. **IF: 2.244**

4. Buzas K, Megyeri K, Hőgye M, Csanády M, **Bogáts G**, Mándi Y. Comparative study of the roles of cytokines and apoptosis in dilated and hypertrophic cardiomyopathies. Eur. Cytokine Netw. 2004; 15(1): 53-9. **IF: 1.747**

5. **Bogáts G**, Kovács GS, Rudas L. Replacement of an aortic Starr-Edwards ball valve prosthesis 28 years after implantation. Circ. J. 2004; 68(11): 1093. **IF: 1.797**

PUBLICATIONS RELATED TO THE SUBJECT OF THE THESIS

1. Jost N, Virág L, Bitay M, Takács J, Lengyel C, Biliczki P, Nagy Z, **Bogáts G**, Lathrop DA, Papp JG, Varró A. Restricting excessive cardiac action potential and QT prolongation: a vital role for IKs in human ventricular muscle. Circulation 2005; 112(10): 1392-9. **IF: 12.563**

2. Opincariu M, Varró A, Iost N, Virág L, Hála O, Szolnoki J, Szécsi J, **Bogáts G**, Szenohradszky P, Matyus P, Papp JG. The cellular electrophysiologic effect of a new amiodarone like antiarrhythmic drug GYKI 16638 in undiseased human ventricular muscle: comparison with sotalol and mexiletine. Curr. Med. Chem. 2002; 9(1): 41-6. **IF: 4.409**

3. Virág L, Iost N, Opincariu M, Szolnoky J, Szécsi J, **Bogáts G**, Szenohradszky P, Varró A, Papp JG. The slow component of the delayed rectifier potassium current in undiseased human ventricular myocytes. Cardiovasc. Res. 2001; 49(4): 790-7. **IF: 4.552**

3. INTRODUCTION

The developments in the 20th century revolutionized medical therapy and diagnostics in cardiac surgery. The trends inevitably gravitated toward a greater capacity to relieve suffering and to prolong life for patients afflicted with cardiac diseases. Although the new specialty was built upon and worked closely with many related fields, it also evolved its own surgical techniques and methods of patient care and research. In a relatively young area of expertise, where techniques are changing rapidly, the innovative approach always brings forth a range of advances. Indeed, milestones were reached very quickly, demonstrating the highly innovative nature of the field (Westaby S 1998):

1923: Eliott Cuttler performed closed mitral commissurotomy.
1938: Robert E. Gross performed the first patent *ductus arteriosus* ligation (Gross RE 1939).
1953: John Gibbon performed the first successful human open-heart operation, using a heart-lung machine (Gibbon JH Jr. 1954).
1956: Walton Lillehei performed the first surgical correction of mitral valve insufficiency.
1961: Albert Star performed the first mitral valve replacement.
1962: Donald Ross performed the first published clinical homograft of the aortic valve replacement (Ross DN 1962).

Open-heart procedures became widespread in the 1950s, and the Medical University of Szeged took part in the progress, played decisive roles in the developments and became a regional center of education, research and health care in cardiac surgery. The first heart operation here was carried out in June 1955 by Gábor Petri with the assistance of Imre Littmann and Antal Temesvári, both from Budapest. The first open-heart operation was performed in 1965.

Today the Cardiac Surgery Department of the Medical School at Szeged University serves the South-east region of Hungary with its approximately 1,400,000 inhabitants. Its activities have increased by 250% during the past decade (Figure 1). The Department is the only center in Hungary dedicated to performing all types of adult and pediatric procedures, including neonatal heart surgery and cardiac transplantation.

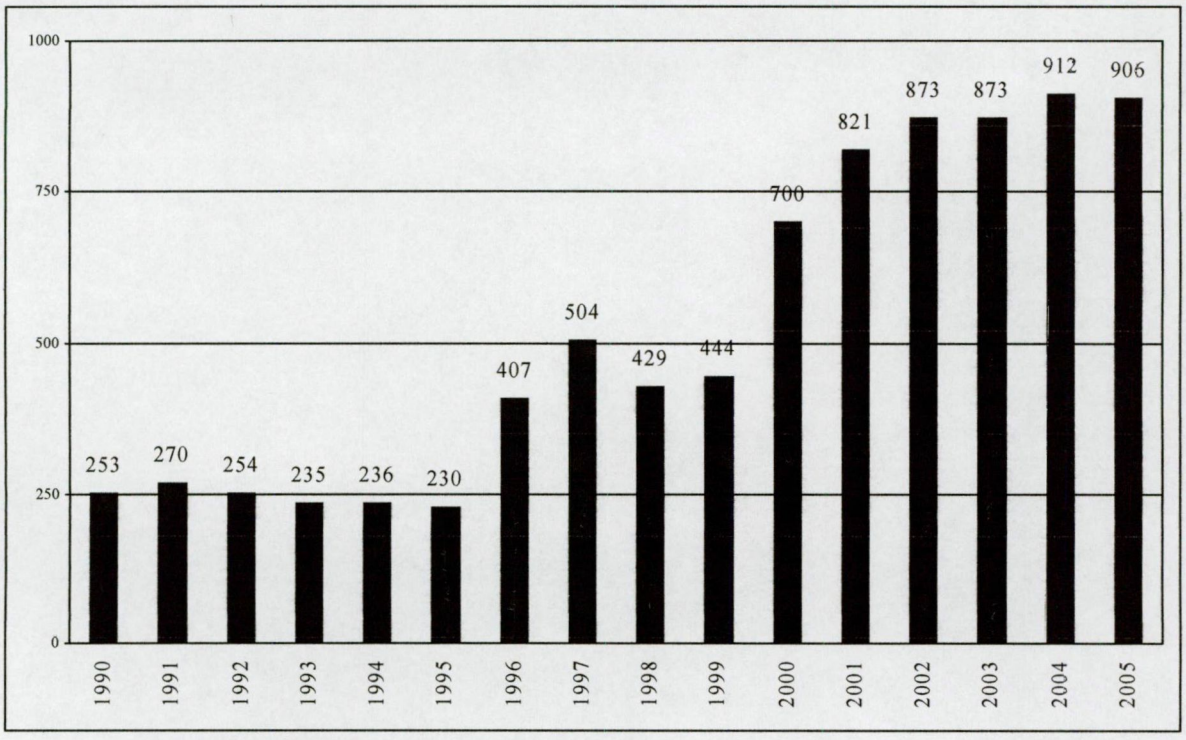

Figure 1. Cardiac surgical activity in Szeged between 1990 and 2005

From January 01, 2000 to December 31, 2005, a total of 5086 open-heart or beating-heart coronary bypass operations were performed at our institution. During this period, the Department did not receive any outside development assistance. With unchanging facilities, we were forced to find and introduce innovative methods to follow the expanding challenges and increasing requirements of our patients.

4. AIMS

The Department of Cardiac Surgery has preserved its clinical data since the 1960s. Information for the institutional database was collected prospectively and analyzed retrospectively to identify eligible patients. The goal of this database was to characterize the epidemiology of heart diseases and to identify risk factors for postoperative complications. Additionally, we have a historical basis as concerns the development of innovative surgical treatments that set the standards in pediatric cardiac surgery. This tradition is continuing, as evidenced by some of our more recent achievements.

Health care is always enhanced by medical education and research. Cardiac surgery necessitates the efforts of team, a coalition of cardiologist, anesthesiologist and surgeon, with the major involvement of supporting specialties. Advances in surgical techniques and knowledge relating to the cardiac function have made heart surgery the first-line procedure for

cardiovascular diseases. As part of an academic medical center, and complementing the complex surgical techniques, the Department of Cardiac Surgery has participated in and acquired experience in clinical research as well. The operating room is a dynamic environment, facilitating the rapid exchange of ideas and innovations, and creates an optimal environment for collaboration. The purpose of this thesis is to discuss and summarize two main aspects of the above possibilities: **(1)** our efforts to improve surgical techniques and technologies (*"clinical core"*), and **(2)** serving as a start-up point for complementary basic research in cardiology (*"basic science core"*).

5. PART I. Clinical core. The possibilities of technical innovations in cardiac surgery

5.1. Innovations in coronary heart disease surgery

In 1967 Kolessov, a Soviet surgeon reported on his experience with mammary artery–coronary artery anastomoses for treatment of angina pectoris. (Kolessov VI 1967). Operations were performed through a left thoracotomy without ECC or preoperative coronary angiography. Since René Favaloro performed the first coronary artery bypass operation based on coronary angiography using ECC in 1968, coronary artery bypass graft (CABG) surgery has become the benchmark of coronary revascularization. (Favaloro RG 1968). However, a cardiopulmonary bypass involves a range of potential complications, including a whole-body inflammatory response, an endothelial dysfunction, arrhythmias, stroke, neuropsychological disorders, multiorgan failure, great vessel damage and coagulopathy. The mortality and morbidity of CABG surgery are mainly attributed to the use of CBP, global cardiac arrest, hypothermia and manipulation of the ascending aorta.

In an attempt to avoid the deleterious effect of the CBP, the *off-pump* technique (OPCAB) has been increasingly used, and has become the predominant method for coronary revascularization in our practice (Figures 2 and 3). Initially, OPCAB was established as an alternate means of applying a less traumatic procedure to a selected group of patients, but today this technique can be applied to the whole population requiring coronary surgery. With the use of advanced tissue stabilizers and heart-positioning methods, complete revascularization can be achieved (Figure 4). Application of an aorto-coronary shunt prevents myocardial ischemia during suturing of the anastomoses. Further, the extended use of intra-aortic balloon counter-pulsation prevents the hemodynamic instability when the heart is dislocated, and reduces the incidence of perioperative complications.

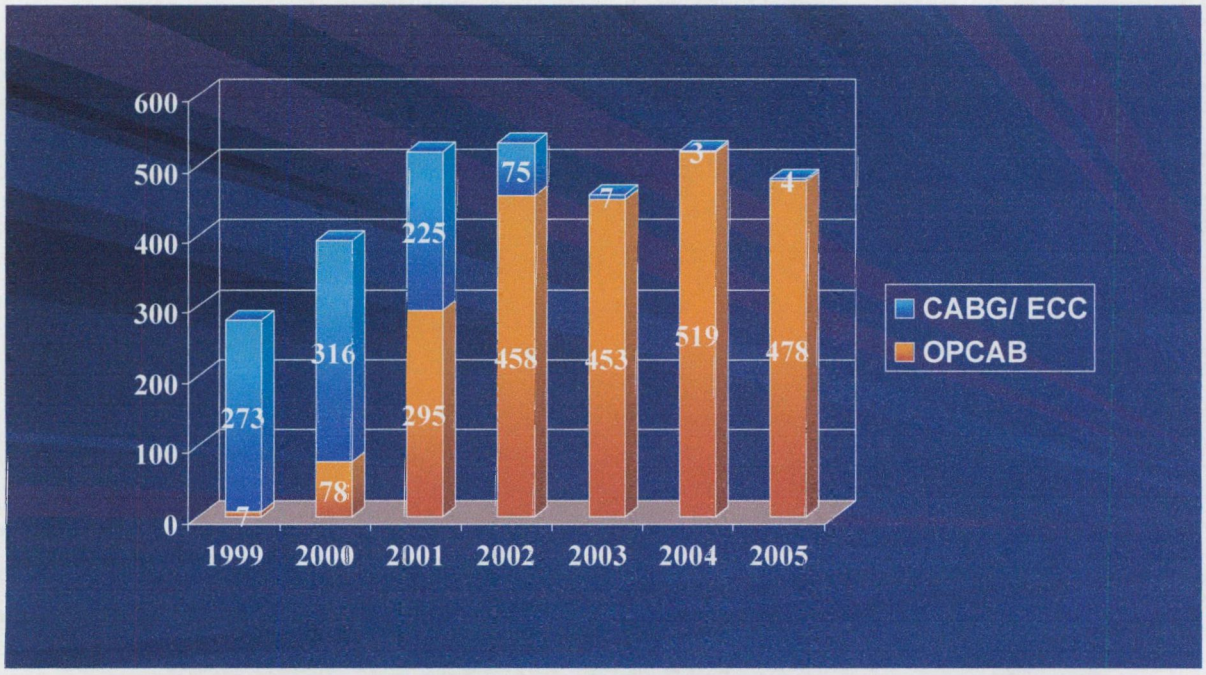

Figure 2. *Off-pump* technique (OPCAB) and coronary artery bypass graft operations (CABG) in Szeged. From January 01, 1999 to December 31, 2005, a total of 3191 coronary bypass operations were performed.

During the period 2003-2005, only 14 of 1464 patients had an ECC intervention, 8 of them electively (due to extreme preoperative hemodynamic instability) and conversion was necessitated in 6 cases during surgery following incidences of arrhythmia or myocardial failure. Actually, the OPCAB technique is used in more than 99% of isolated coronary cases, with low mortality (1.46% in 2005) and morbidity rates (Figure 3).

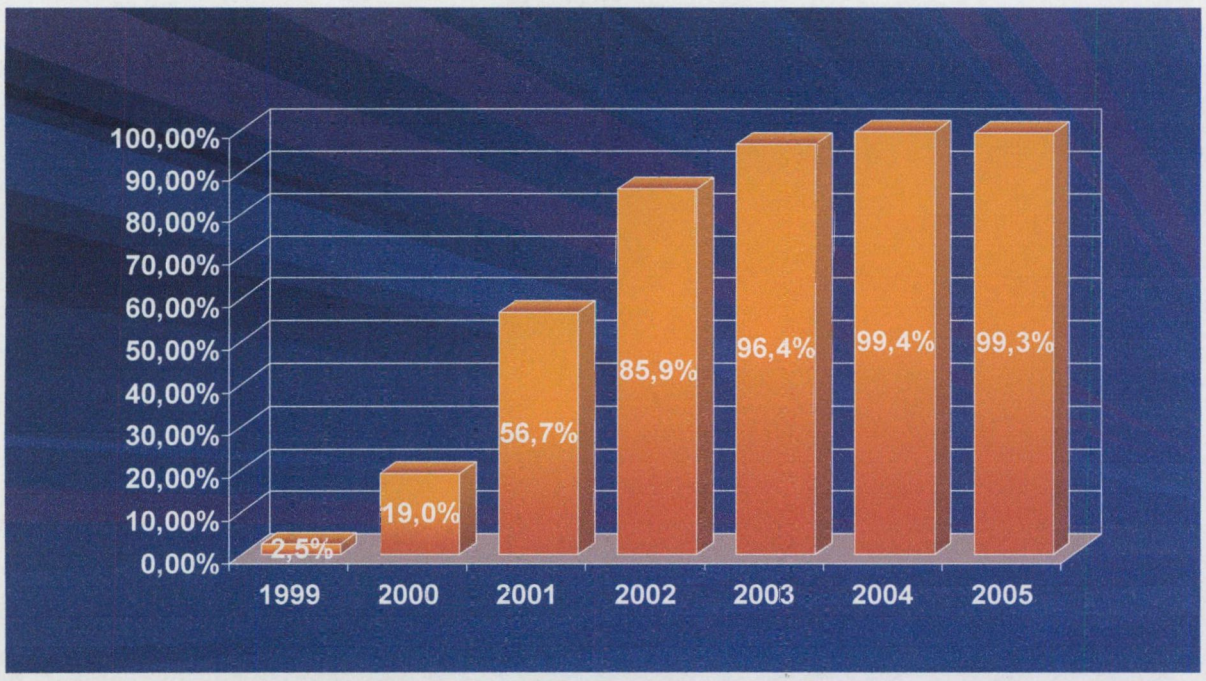

Figure 3. The proportion of OPCAB/CABG operations between 1999 and 2005 in Szeged

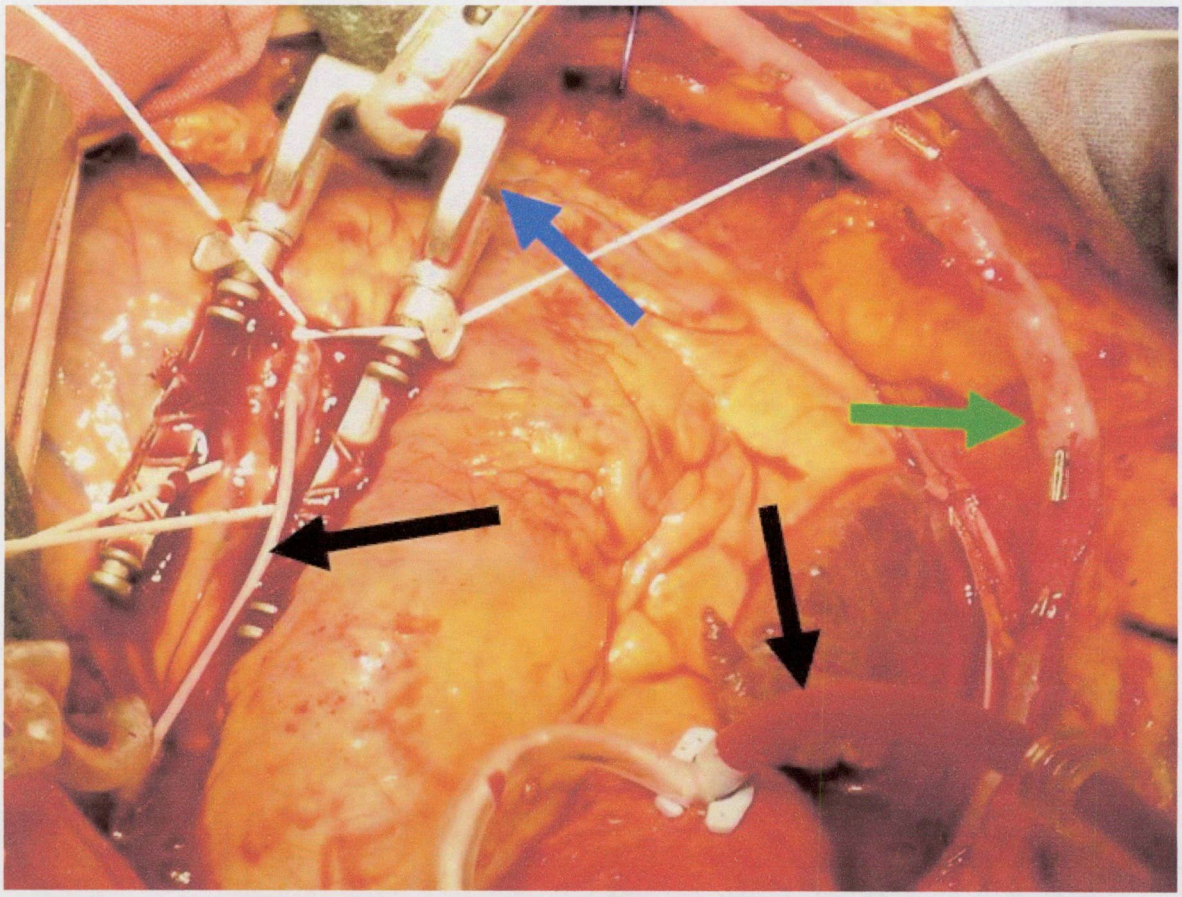

Figure 4. Aorto-coronary shunt introduced into the left anterior descending coronary artery encircled by elastic sutures (black arrows). The IABP is inserted through a homograft saphenous vein anastomosed to the anterior surface of the ascending aorta (green arrow). The blue arrow shows the OPCAB tissue stabilizer.

5.2. Innovations in valve surgery

Historically, the Starr-Edwards mechanical valve is considered to be one of the most long-lived legacies of the early prosthetic valve era. The ball valve was introduced into clinical practice in 1961 (model 1000) and in the following years a serious complication, *'ball variance'*, was observed in a high percentage (20-67%) of the early models. Ball variance was defined as a "valvular malfunction" resulting from physical and chemical alterations in the silastic poppets, which included an increased diameter of the ball that impinged on the cage struts, grooving, cracking, a decreased diameter, fragmentation of the poppet, or changes in the poppet core with the formation of fluid lakes, and the result was a valve dysfunction (*i.e.* obstruction and/or regurgitation, silastic embolization, intravascular hemolysis leading to severe anemia, and finally escape of the ball from the cage) (Ghosh PK *et al.*, Hylen JC *et al.*). Modification of the production of the silastic ball (some alterations were made in the composition of the silastic material, and the curing process was changed) considerably increased the durability in the new models (1200 and 1260).

In 2004, Tayama and coworkers presented a case report on a patient who had had an aortic Starr-Edwards ball valve (model 1260) implanted 28 years previously and which had functioned normally over the years, without any signs of hemolytic anemia (Tayama K *et al.*). Owing to progressive mitral valve disease, the patient's mitral valve was replaced and concomitantly his normally functioning aortic prosthesis was removed and changed to a St Jude valve. The explanted Starr-Edwards prosthesis revealed no abnormality, and there was no further occurrence in ball variance after 1965 in the modified prosthesis. However, fatal complications were reported later with the Cross-Jones disc valve prostheses, which had a silastic disc prepared by using the same technology as the ball of the Starr-Edwards model 1260 valve (it had undergone a proper curing process).

In Szeged, 145 Cross-Jones valves were implanted into 121 patients in the late 1960s. In 90 of those prostheses the metal seat was covered with a dacron cloth to enhance encapsulation (it was thought at that time that this would prevent thromboembolism); the remaining 55 prostheses were uncovered (50 aortic and 5 mitral). Most of the cloth-covered prostheses developed serious complications, resembling those of ball variance: frequent thromboembolism, serious hemolysis, and escape of the disc from the prosthesis in 7 cases, with resulting death.

A thorough study of the removed prostheses (at autopsy or reoperation) revealed that the encapsulated cloth had ruptured and rolled over, and the resultant uneven surface of the seat hindered the proper closure of the disc, thus causing a serious insufficiency. In addition, hitting the uneven surface of the seat caused continuously increasing wear of the disc, with a subsequent diminution of size and finally escape of the disc from the cage. Although the cause and mechanism of the deterioration of the disc were different from those of the silastic ball in the original 'ball variance', the result was very similar. In contrast, the discs in the uncovered prostheses did not show any signs of wear, even after decades, and valve dysfunction did not occur.

Recently, one of our patients died of an unrelated cause at the age of 79 years. Owing to severe aortic insufficiency, his aortic valve had been replaced 35 years previously with an uncovered Cross-Jones prosthesis; postoperatively he lived a full life, including cycling and doing heavy agricultural work. His prosthesis was functioning normally without any sign of wear of the disc (Kovacs GS *et al.*). That case also proves that well-prepared (*i.e.* properly cured) silastic discs and balls can function normally, without any alteration, over decades, as in the case presented by Tayama and colleagues (Tayama K *et al.*).

During the past decade, the total volume of heart valve surgery has slightly increased, a predominance of aortic valve replacement for degenerative calcific aortic stenosis. The incidence of rheumatic mitral valve disease has significantly decreased due to the beneficial effect of antibiotic prophylaxis.

Between 2000 and 2005, 1724 patients underwent heart valve operations in Szeged, targeting 2155 orifices. 1781 mechanical valves, 376 xenograft valves and 106 homograft valves were implanted. We performed 32 aortic valve replacements using pulmonary valve autografts (Ross procedure) and 400 valvuloplasties. Figure 5 demonstrates the tendency toward the increasing use of biological valves and valve-preserving surgery.

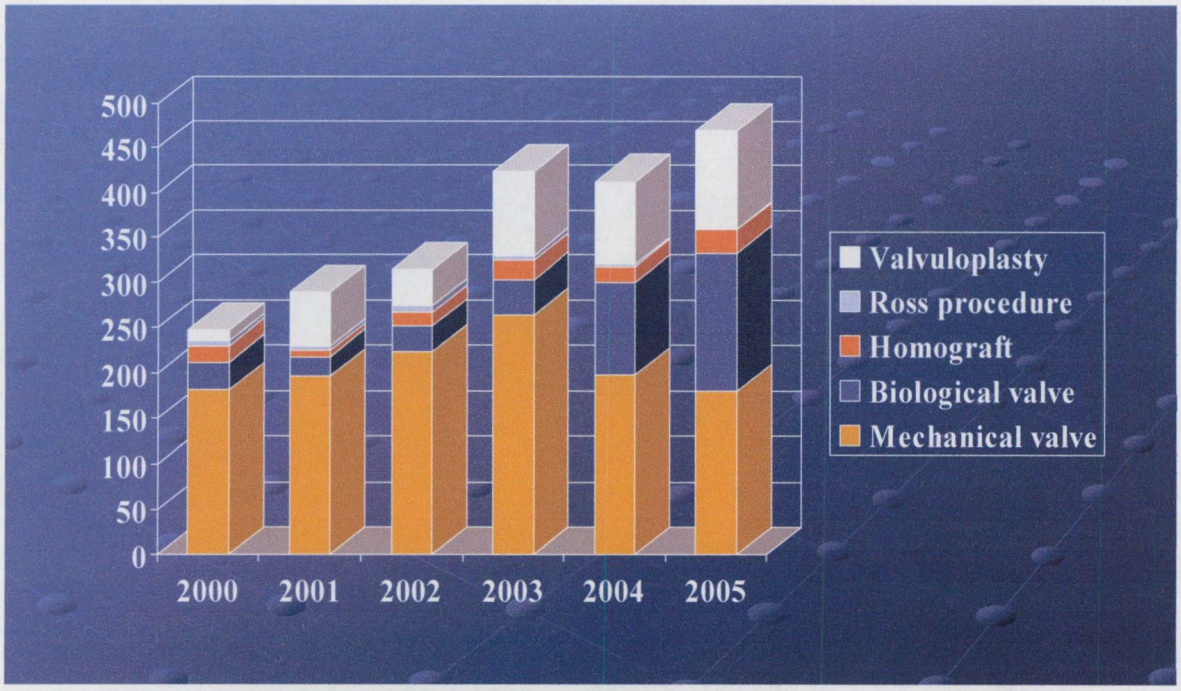

Figure 5. Valve surgery in Szeged between 1999 and 2005.

5.2.1. Homografts

The first homograft valve was implanted by Gordon Murray in 1955. The donor valve was taken from a cadaver and preserved in physiological saline solution at 4 °C for 36 hrs. It was then inserted into the patient's descending aorta without CBP. Six years after the operation, the patient was asymptomatic.

The first orthotopic, subcoronary aortic homograft implantation was performed by the Toronto group in 1961 (Westaby S 1998). During the early 1960s' different types of mechanical valve substitutes were developed, making homografts less popular among the majority of cardiac surgeons (Figure 6).

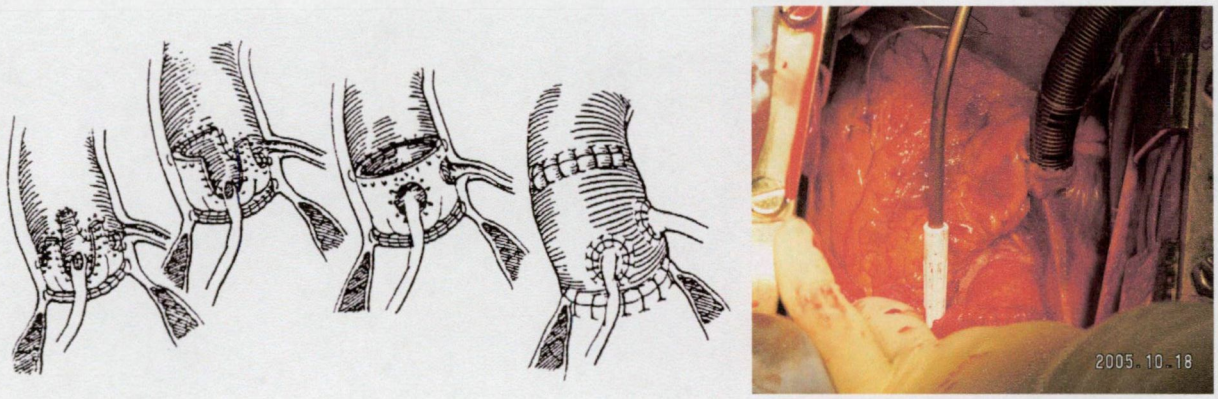

Figure 6. Different techniques for implanting a homograft aortic valve (left). A homograft implanted by using the total root replacement technique (right).

Crude homograft preservation techniques caused early clinical failure, whilst mechanical prostheses offered the prospect of indefinite durability. Access to human cadaveric material was limited and a valve of appropriate size was not always available when needed. With the development of gentle preservation techniques and cryopreservation the performance of aortic homografts improved and a stock of deep-frozen valves was available for installation.

Figure 7. Laboratory equipment for preserving homografts.

A laboratory for harvesting, preserving and storing homografts was established in Szeged in 1989 (Figure 7). Since 1990, homografts are cryopreserved and stored in liquid nitrogen vapor at -196 °C. During the last 6 years, these self-processed homograft valves were used for aortic valve replacement in 106 cases, and for the Ross procedure in 32 patients,

while 12 pulmonary valve replacements and 16 corrections of a right ventricular outflow tract obstruction were performed.

5.2.2. Ross procedure

The Ross procedure solved some of the inherent drawbacks of mechanical and biological (xenograft) valve substitutes (Ross DN 1967). Mechanical valves require lifelong anticoagulation, with the risk of thromboembolic and bleeding complications. Xenograft valves, however, degenerate in the long run and necessitate redo surgery. Donald Ross performed his milestone operation in 1964, replacing a diseased aortic valve and the root of the aorta with the patient's own pulmonary outflow tract containing the right ventricular infundibulum, the pulmonary valve and a part of the main pulmonary artery. The pulmonary outflow tract defect was then corrected by a pulmonary homograft. Since 1999, 32 patients have been operated on by this technique at the Department, including both pediatric and adult cases, comprising the largest series of Ross procedures in Hungary.

5.2.3. Minimal access mitral valve surgery

Minimal access surgery provides a good means for mitral valve replacement and valvuloplasty in selected cases (Figure 8). This technique was introduced into clinical practice in Szeged in 2004, and 39 operations were performed up to the end of 2005, without any mortality or serious complications.

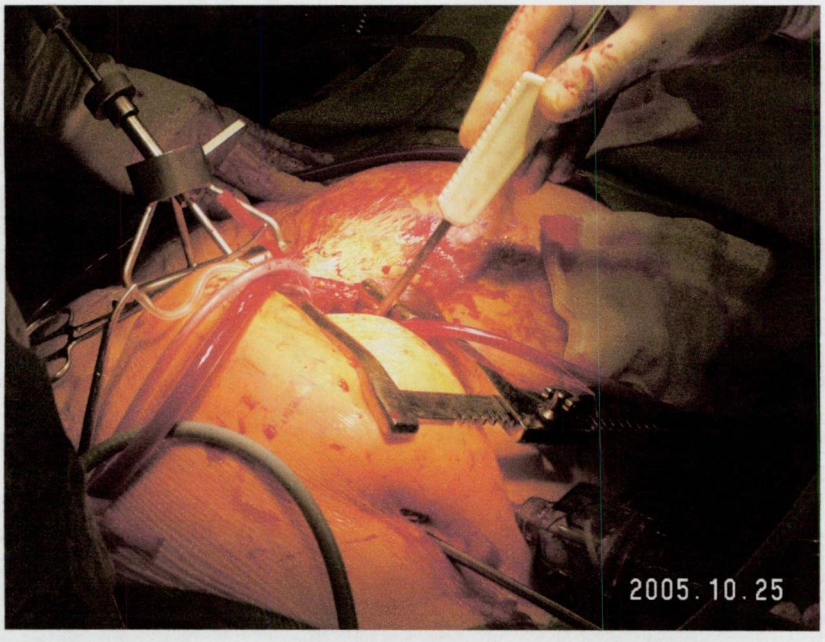

Figure 8. Minimal access surgery for mitral valve replacement

5.2.4. Extra-anatomic placement of injectable heart valve

In cases of a porcelain aorta, access to the aortic valve is impossible due to the extensive calcification. In such cases, the only solution currently available is an apico-aortic valved conduit (Gemmie JS *et al.* 2006). For this purpose, we used a reversed injectable pulmonic heart valve. The operation was carried out by inserting the self-expanding valve into the apex of the left ventricle, and a vascular graft was used to connect the apex with the ascending aorta. As far as we are aware, this was the first application of this type of valvular prosthesis for this field of indication (Figure 9).

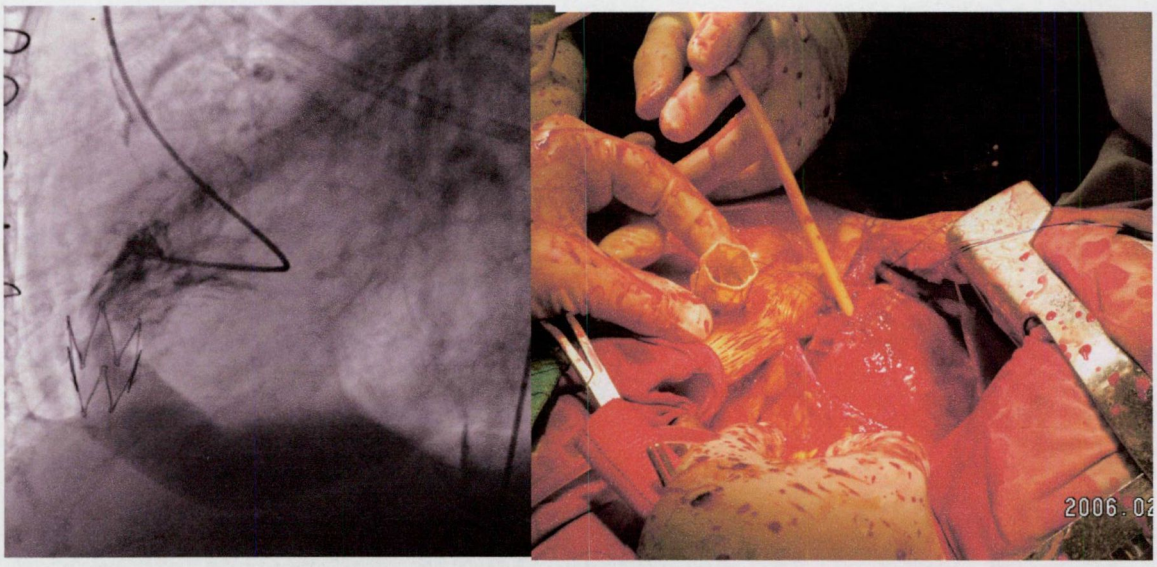

Figure 9. Extra-anatomic placement of injectable heart valve

5.3. Innovations in congenital heart surgery

Congenital cardiac disease (CCD) is caused by structural problems of the heart that affect nearly 1% of newborns. Approximately 50-60% of these defects are diagnosed shortly after birth, while the remainder are often undetected until later in life. Surgical results for CCD have improved dramatically over the last 25 years. The advances in such areas have significantly decreased the morbidity and mortality once common in neonates, young infants, children, adolescents, and adults. The conditions that we treat most often in the various age groups include: transposition of the great arteries (TGA), tetralogy of Fallot (TOF), ventricular septal defects, atrial septal defects (ASDs), total anomalous pulmonary venous return, aortic coarctation, double-outlet right ventricle (DORV), atrioventricular canal, patent ductus, single ventricle (SV) and pulmonary atresia (PA), Ebstein anomaly and vascular ring.

5.3.1. Right ventricular outflow tract reconstruction

For right ventricular outflow tract reconstruction, several methods are used. In rare cases, when transannular augmentation of the obstruction is required, a pulmonary homograft patch containing one of the cusps of the pulmonary valve is implanted. The orientation of the patch permits the closure of the monocusp against the supraventricular band (Figure 10). This technique has been successfully used in 4 infants so far.

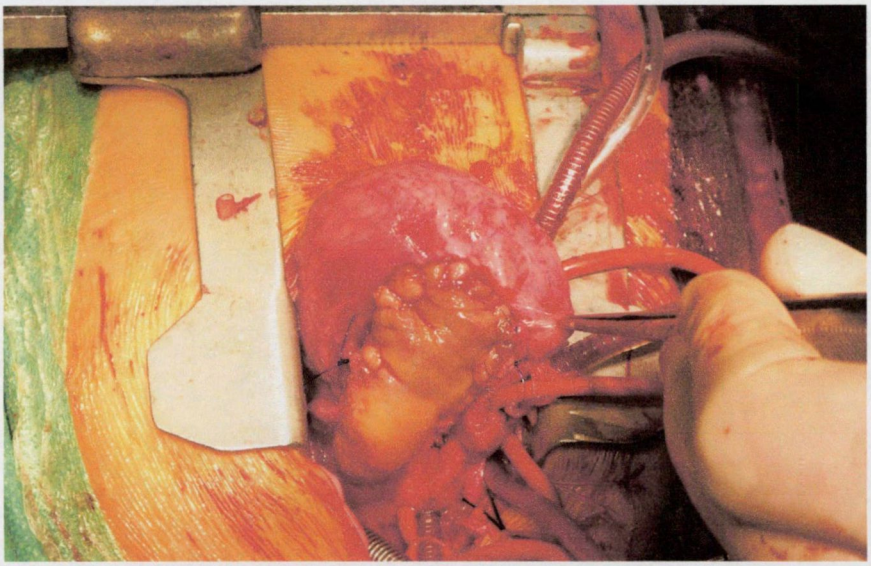

Figure 10. Right ventricular outflow tract reconstruction with a homograft

5.3.2. Minimal access atrial septal defect (ASD) closure

In many cardiac surgical centers, a standard access technique for ASD closure is midline sternotomy or right anterior thoracotomy. We applied a novel method of right axillary minithoracotomy with excellent cosmesis. The operation can be performed without groin cannulation by using special venous and arterial cannulae through the incision (Figure 11).

Figure 11. Right axillary approach for the closure of an ASD

5.3.3. Homografts for the creation of modified Blalock-Taussig shunts

Modification of the original Blalock-Taussig shunt (1945) by interposing a conduit between the subclavian and pulmonary arteries (Klinner VW *et al.*, De Leval MR *et al.*) has several advantages: it is technically simpler because **a)** less dissection is needed, **b)** the blood flow to the respective arm is not jeopardized because the subclavian artery is not sacrificed, and **c)** there is no subclavian steal effect (Watkins MT *et al.*). With the introduction of expanded polytetrafluoroethylene grafts (Gazzaniga AB *et al.*) (Gore-Tex, W.L. Gore & Assoc, Flagstaff, AZ; Impra Medica SA, Geneva, Switzerland), smaller conduits (4 to 6 mm) can be safely used without increasing the thrombotic obstruction rate (De Leval MR *et al.*, Ullom RL *et al.*, Ilbawi MN *et al.*). As the orifice of the subclavian artery serves as a flow regulator over-perfusion of the lungs can be avoided, and an increase in shunt flow can be expected with growth (De Leval MR *et al.*). Thus, the procedure can be applied even to small infants, and has become the most common shunt operation (Ullom RL *et al.*, Ilbawi MN *et al.*). Despite the obvious advantages of the polytetrafluoroethylene conduits, however, they are not free of special complications. Lengthy oozing of blood or serous leakage from the stitch holes or through the graft can occur (LeBlanc J *et al.*) and, because of their relative stiffness, the grafts may kink if wrongly positioned.

Until 1989, Impra or Gore-Tex was used almost exclusively at this institution for modified Blalock-Taussig shunts. However, the appearance of a life-threatening late complication in 2 consecutive patients discouraged their further use. In 2 patients, a pseudoaneurysm developed at the site of the proximal anastomosis after infectious complications several years after the shunt operation; in 1 of these cases, it occurred 1 year after a successful operation and ligation of the Impra shunt.

Having employed some saphenous vein allografts successfully as coronary artery bypass grafts, we decided to use homograft veins left over from previous CABGs for the creation of modified Blalock-Taussig shunts, first preferably in infected patients. Later, the preliminary good results encouraged us to use them more extensively. The overall aims were to investigate the fate of the homograft veins over a 6-year period.

Harvesting of saphenous vein allografts

Saphenous veins were collected during CABGs by using a no-touch technique. They were then stored in heparinized autologous blood at room temperature until completion of the revascularization. Unused vein segments were next washed with sterile saline solution and distended temporarily to 80 to 100 mm Hg. Their external diameter was measured with sterile caliper squares, and they were then placed for 24 h at 4 ^{o}C in a modified Hank's solution

containing low concentrations of antibiotics (cefotoxin, 140 μg/ml; lincomycin, 120 μg/ml; polymyxin B, 100 μg/ml; vancomycin, 50 μg/ml, and amphotericin B, 25 μg/ml) (Strickett MG *et al.*).

In the first part of the study all vein segments were stored at 4 °C in the modified Hank's solution for 6 weeks at the longest; if they were not used by then, they were discarded. Veins stored in Hank's solution were used in 8 patients after storage for 3 to 38 days (average: 22.4 days) (Davies AH *et al.*). In the second part of the study, the vein segments were cryopreserved after the 24-h sterilization procedure in Hank's solution. They were placed in a solution containing 10% dimethylsulfoxide and 10% human albumin, and cooled down to -196 °C in a computer-controlled Sy-Lab model 1510 (Sy-Lab GmbH, Parkersdorf, Austria) deep-freezing apparatus. The cooling rate was even at 1 °C per min; thus, the cells were not damaged and the tissue structure was well preserved (Figure 12) (O'Brien MF *et al.*, Armiger LC *et al.*).

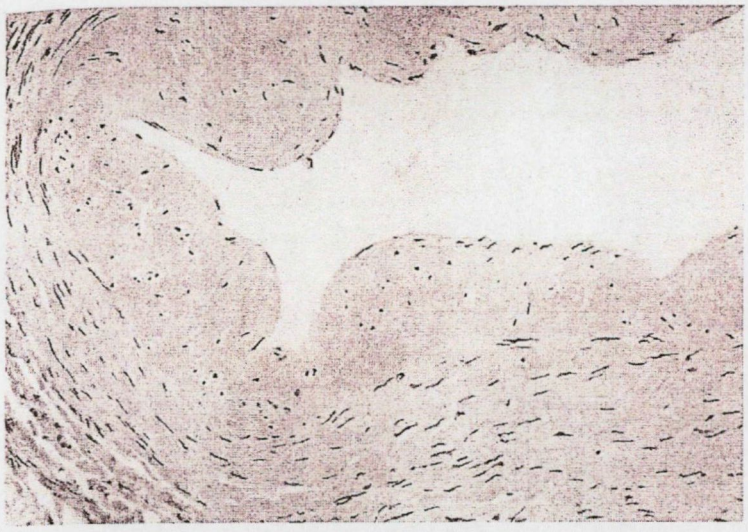

Figure 12. Microscopic view of a cryopreserved saphenous vein after thawing. The wall structure is normal (HE staining; x180 before 23% reduction).

Tissue samples that underwent the same procedures were taken from the grafts both at harvesting and at operation, and were sent for bacteriologic and histologic studies. Similarly, blood was taken from the donors for serologic tests for hepatitis, human immunodeficiency virus, cytomegalovirus, and syphilis. If any of these tests turned out to be positive, the grafts were not used. The allografts were then stored in liquid nitrogen vapor; thus, they could be preserved practically indefinitely. Grafts stored in Hank's solution were rinsed thoroughly in saline solution at the operating table; the cryopreserved tissues were thawed in a water bath at

40 °C, and the dimethylsulfoxide was then rinsed off thoroughly in several changes of saline solution before implantation.

Modified Blalock-Taussig shunt operations using allograft saphenous veins have been performed in 23 patients (Table 1). In 8 patients vein segments preserved in Hank's solution were applied; in 15 others, cryopreserved tissues were used. No effort was made to take blood groups or tissue typing into consideration in the selection of the respective grafts. The ages of 10 female and 13 male patients varied from 6 days to 18 years (average: 38.7 months), and their weight was between 2.29 and 41 kg (average: 10.36 kg). The diagnosis was TOF in 12, PA in 6, DORV with pulmonary stenosis (PS) in 2, and SV with PS in 3 patients. In older children or adults, the indications for shunt operation were extreme cyanosis with a small left ventricle and a low ejection fraction or an uncorrectable lesion. Previous palliative interventions had been performed in 3 patients: in 2, a closed Brock operation had been carried out, and in 1 an original Blalock-Taussig operation was performed in infancy, and after its occlusion percutaneous balloon valvuloplasty was performed repeatedly.

Table 1. Data on patients with modified Blalock-Taussig shunts using allograft saphenous veins.

Patient no.	Age (mo)	Follow-up (mo)	Weight (kg)	Diagnosis	Shunt (mm)	ml/min	Shunt flow ml/min/kg
1	96	71	19.00	PA	5.5		
2	12	67	6.14	TOF	4.0		
3	1	66	3.30	PA	4.5		
4	0.25	+3 days	2.89	PA	4.5		
5	9	60	7.90	TOF	4.5	950*	
6	1	+11 mo	2.70	PA	5.5		
7	1	57	2.60	PA	5.0		
8	10	+11 mo	4.12	TOF	5.0		
9	26	52	9.70	TOF	5.5		
10	26	52	5.45	TOF	4.0		
11	23	51	9.50	TOF	5.5		
12	17	50	10.00	TOF	5.5		
13	49	+1 day	14.30	DORV,TGA,PS,DX	6.5		
14	6	40	6.40	DORV, PS	5.0		
15	1	40	2.29	PA	5.0		
16	11	35	6.40	TOF	4.5	500	78
17	6	31	5.56	TOF	4.0	430	77.3
18	62	25	14.30	SV,PS	8.0	1.354	94.7
19	7	20	7.20	TOF	6.0	522	72.5
20	56	19	13.60	TOF	6.5	1.200	88.2
21	180	18	26.00	SV,PS	6.0	1.800	69.2
22	216	17	41.00	TOF	6.0	760	18.5
23	74	12	18.00	SV,PS	6.0		

* Measured at corrective operation 25 months after the shunt operation. DORV = double-outlet right ventricle; DX = dextrocardia; PA = pulmonary atresia; PS = pulmonary stenosis; SV = single ventricle; TGA = transposition of the great arteries; TOF = tetralogy of Fallot.

Operative technique

The operative technique did not differ from that generally used in the creation of a modified Blalock-Taussig shunt: on 20 occasions the left and in 3 cases the right subclavian arteries were used. On clamping-off of the subclavian artery, 1 mg/kg heparin was given intravenously, which was not reversed later. A longitudinal incision was made in the subclavian artery near its origin, and a cobra-head anastomosis was performed with the obliquely cut saphenous vein. A graft the size of the subclavian artery or larger was chosen if

available. For the distal anastomosis, the left or right main pulmonary artery was incised longitudinally (18 patients) or transversely (5 patients). The anastomoses were performed with a single running suture of 6/0 or 7/0 Prolene (Ethicon, Somerville, NJ, USA). The lengths of the venous segments ranged from 30 to 80 mm (average: 44 mm), and their diameter was between 4 and 8 mm (average: 5.36 mm).

Blood flow measurements

Blood flow through the shunt was measured with an ultrasonic flowmeter (Transonic AT 107; Transonic Systems, Ithaca, NY) in 7 patients at the completion of the shunt, and in 1 patient at reoperation. The method was previously validated (resistances were measured during coronary surgery in over 500 saphenous grafts. The results were highly reproducible and comparable; Kovacs GS *et al.* 1990).

Results

There was no operative death in the 23 operations. Technically the operations were easy and simple; because the allografts are pliable and soft, they were easy to handle and to sew. There was no bleeding or serious leakage after completion of the anastomoses. Blood flow through the shunt was measured with an ultrasonic flowmeter (Transonic AT 107; Transonic Systems, Ithaca, NY) in 7 patients at the completion of the shunt, and in 1 patient at reoperation; the flows were between 430 and 1,800 ml/min (see Table 1). Arterial blood samples were taken before and after the shunt operation: the average arterial oxygen tension rose from 35.22 ± 4.88 mm Hg to 45.57 ± 6.96 mm Hg as a result of the increased pulmonary flow.

There were two early deaths, 1 and 3 days postoperatively, which were unrelated to the function of the shunt. One patient, a 6-day-old neonate with PA, was operated on for severe hypoxia as an emergency. Although the shunt functioned well and the patient became much less cyanotic, she never recovered, and she died of cerebral damage on the third postoperative day. Another 4-year old patient had a DORV with a VSD and ASD, severe PS, and dextrocardia. An original left-sided Blalock-Taussig shunt was performed at 6 months, which occluded years later. He underwent two unsuccessful pulmonary valve balloon dilations, and finally the modified Blalock-Taussig shunt was performed on the right side for severe hypoxia as an emergency operation. The shunt functioned well postoperatively, but cerebral damage developed, and the patient died 14 h after the operation. In both cases, the vein segment was completely patent, and no kinking or thrombus formations were observed.

On histologic examination both 1 and 3 days after implantation the original structure of the venous wall had disappeared; it had become homogenous eosinophilic, loose tissue containing no cells, except some in the adventitial layer. Signs of rejection or foreign body reactions were not observed (Figure 13).

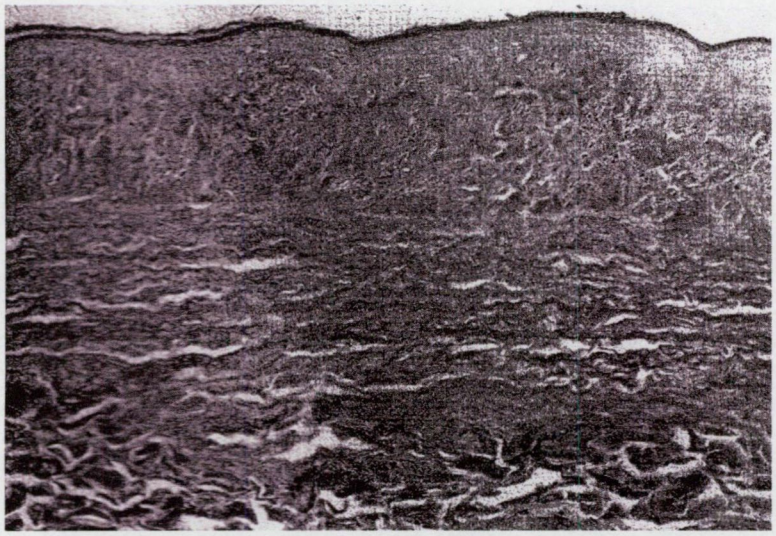

Figure 13. Histologic picture of a cryopreserved venous allograft implanted 14 h before death, and recovered at autopsy. The wall is homogenous and eosinophi1ic; no intimal and medial cells can be found (HE; X180 before 23% reduction).

Two patients died 11 months after the operation in remote hospitals after respiratory infection. One of them had received a graft kept in Hank's solution only, and the other a cryopreserved vein. In both cases the shunt was patent with no apparent macroscopic change. The two grafts appeared similar on histologic examination: the wall of the veins was still somewhat homogenous, with a few non-inflammatory cells, which presumably originated from the recipient. The luminal surface was thinly and non-continuously covered with endothelial-like neointimal cells (Figure 14).

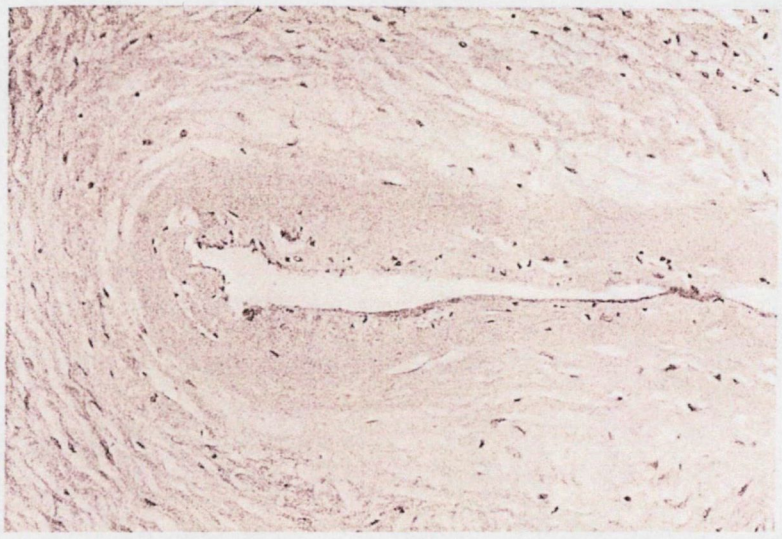

Figure 14. Histologic picture of a cryopreserved venous allograft implanted 11 months before death (HE; x180 before 23% reduction).

Graft occlusion occurred in only 1 patient (patient 17) in the early postoperative period. This was very likely due to kinking of a too long segment of cryopreserved vein 4 mm in diameter. Since the outflow obstruction was situated mainly at a valvular level, a closed Brock operation was performed shortly afterward as a further palliation, with good results.

No medical therapy was applied either in the early or in the late postoperative period for the protection of the shunts: no immunosuppressive drugs, anticoagulants, or antiplatelet drugs were given. All patients were routinely followed up during an average period of 41.21 months, the longest follow-up being almost 6 years. All patients living with their shunts are well, their growth was normal, they are slightly cyanotic, and their hematocrit is between 0.39 and 0.50. Except for 1 patient whose graft had occluded, no other patient needed further palliation. On auscultation, a strong continuous systole-diastolic murmur can be heard. On echocardiography, a high shunt flow can be demonstrated in all patients.

Angiocardiography was performed in 9 patients: the shunts were all functioning well, and no stenosis, kinking, or aneurysm formation could be observed (Figure 15).

Figure 15. (patient no. 2) Aortic arch angiogram 4 years after a left-sided modified Blalock-Taussig shunt using a saphenous allograft. The shunt flow is filling both pulmonary arteries.

Final corrective operation was performed in 4 patients (patients 2, 5, 7 and 9) from 25 to 55 months after the shunt operation. There were no complications, and the dissection and ligature of the allograft were similar to those experienced on dissection of the subclavian artery in the original Blalock-Taussig operation. (However, dissection of a Gore-Tex shunt is easier, because of its stiffer nature and lack of tissue adhesion to the synthetic material.) There was no apparent fibrosis or calcification around the allografts. In 2 patients (25 and 55 months after the shunt operation), the grafts were doubly ligated proximally and distally, and a short segment was excised for histologic examination. In both cases, the luminal surface was completely covered with neointima and the medial layer was almost normally populated with cells, presumably of recipient origin (Figure 16).

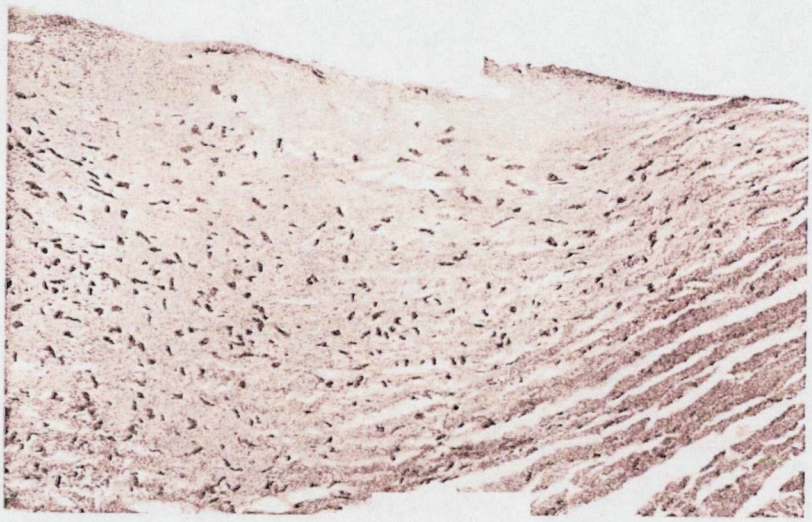

Figure 16. Histology of allograft saphenous vein preserved in Hank's solution and implanted 55 months before the corrective operation, when a segment had been excised from the ligated segment. The luminal surface is covered with neointima, and the medial layer is homogenous (HE; x180 before 23% reduction).

Summary

In cases where appropriate autologous saphenous veins were not available, allografts have been used in the past both in peripheral and in coronary artery operations (Brockbank KG *et al.*, Martin RS *et al.*, Sellke FW *et al.*, Hoover WW *et al.*, Laub GW *et al.*, Vermassen F *et al.*). The results obtained with the allograft, however, have been worse than those after the use of autologous veins (Martin RS *et al.*, Sellke FW *et al.*, Laub GW *et al.*, Vermassen F *et al.*). In operations on CCD, efforts have been made to use biografts for the creation of systemic-pulmonary shunts (Danilowicz D *et al.*, Vincent JG *et al.*, Wojtalik M *et al.*).

The occurrence of life-threatening late infectious complications after the use of expanded polytetrafluoroethylene conduits as modified Blalock-Taussig shunts prompted us to apply allograft saphenous veins instead. In 23 cyanotic patients (1 week to 18 years old) allograft saphenous veins were used for performing Blalock-Taussig shunts from July 1989 onward. Veins stored in Hank's solution were implanted in 8 patients and cryopreserved ones in 15. All patients were followed up regularly up to 15 months. There were two early and two late deaths: none were related to shunt occlusion. Clinical, angiographic, and echocardiographic studies proved that, except for one early occlusion, all shunts were patent and functioning well after an average of 41 months. Donor cells disappeared 1 to 3 days after implantation, and several months after the operation both the wall and the luminal surface of the grafts were repopulated with cells possibly of recipient origin. No difference was found between veins stored in Hank's solution only and cryopreserved grafts as concerns the clinical

outcome and histology. In the present material, the patency rate of the allografts was superior to that reported in coronary artery operations: of 21 allograft saphenous vein segments, 20 were patent at 11 months after implantation (95.2%). This may be due to the different immunoreactivity of infants and small children, or more probably to the much higher flow rates in these short segments.

The results showed that a human saphenous vein allograft is a good alternative biomaterial for the performance of a modified Blalock-Taussig shunt. It is easily available, its use simplifies the operative procedure, and it is devoid of bleeding complications and serous leakage. Being a biomaterial, it is less presumably prone to take part in infectious complications. Ligation of the shunt during the corrective operation should not cause any difficulties either. In the presented material, there were no apparent differences either clinically or histologically between veins preserved in Hank's solution and cryopreserved ones. During the last 6 years, an additional 16 systemic to pulmonary shunts have been carried out with saphenous vein allografts.

6. PART II. Basic science core. The link between clinical practice and research

6.1. Background

Despite advances in the technical management of CCDs, the mortality and morbidity rates of heart failure are expected to increase. In part, this is due to the aging of the population, but it is also a reflection of the success in treating patients with certain types of heart disease, who increasingly survive but are often left with a significant heart dysfunction. Improvement of the quality of life and prognosis for patients with heart failure is an important task for contemporary clinical sciences. Researchers worldwide are striving for a better understanding of heart failure at molecular and cellular levels, and to discover and evaluate new and better forms of heart failure therapy, including drugs, devices and treatment strategies. Similarly, the aim of our experimental work was to provide a basis for collaborative, multidisciplinary investigations aimed at obtaining an insight into the pathomechanism of acquired heart diseases. Several routes were followed, searching for and studying physiological and pathophysiological pathways that may play a role in the evolution of heart failure. The focus of this multi-faceted research effort is to identify molecular targets for future therapies.

Cardiomyopathies are diseases of the heart muscle that often result in a cardiac dysfunction and heart failure, and are the major reason for heart transplantation (Fatkin D *et*

al. 2002). Primary cardiomyopathies are due to inherited gene defects, while the secondary forms can be caused by a great variety of noxious conditions, including ischemia, inflammation, infections and toxic compounds (Fatkin D *et al.*, Richard P *et al.*). Cardiomyopathies have been classified into four groups:

(i) dilated cardiomyopathy (DCM),
(ii) hypertrophic cardiomyopathy (HCM),
(iii) arrhythmogenic right ventricular dysplasia, and
(iv) restrictive cardiomyopathy.

DCM is characterized by a contractile dysfunction and dilatation of the ventricles (Fatkin D *et al.*, Richard P *et al.*). HCM manifests as hypertrophy of the ventricles, with histologicai features of myocyte hypertrophy, myofibrillar disarray and interstitial fibrosis (Fatkin D *et al.*). The chronic biomechanical stress, contractile dysfunction and hypoxia lead to progressive deterioration of the ventricular function and evoke the activation of a complex cascade of compensatory mechanisms to maintain the cardiac function (Colucci WS).

Previous studies have revealed that proinflammatory cytokines are important contributors to the cardiac dysfunction in numerous cardiovascular disorders (Shan K *et al.*, Mándy Y *et al.*). Recent observations have indicated that the pathogenetic role of cytokines in heart diseases is connected with their ability to modulate (*i.e.* induce or inhibit) the process of apoptosis of cardiac myocytes (Marshall D *et al.*). Tumor necrosis factor-alfa (TNF-α) exerts a cardiotoxic effect through different mechanisms. It depresses the contractile functions, promotes left ventricular remodeling, diminishes α-adrenergic inotropic responsiveness, and activates both anti-apoptotic and pro-apoptotic pathways in the myocardium (Finkel MS *et al.*, Krown KA *et al.*, Kubota T *et al.*, Kurrelmeyer KM *et al.*, Song W *et al.*). Interaction between the Fas ligand (FasL) and Fas may also mediate the apoptotic demise of cardiac myocytes under certain pathophysiological conditions, while the normal myocardium and cardiomyocyte cultures exhibit some resistance to Fas-mediated cytotoxicity (Lee P *et al.*, Nishigaki K *et al.*, Wollert KC *et al.* 2000). The TNF-α and Fas pathways of apoptosis have already been implicated in the pathogenesis of myocardial infarction, ischemia-reperfusion injury, cardiomyopathies and congestive heart failure (Marshall D *et al.*, Kurrelmeyer KM *et al.*, Lee P *et al.*, Nishigaki K *et al.*, Wollert KC *et al.* 2000, . Bryant D *et al.*). Interleukin-6 (IL-6) has also been associated with heart diseases, and has been shown to exert a negative inotropic effect, and to regulate hypertrophy and apoptosis (Finkel MS *et al.*, Woller KC *et al.*

2001, Hirota H *et al.*). The gp130, Janus kinase, and signal transducers and activators of the transcription signaling pathway, activated by IL-6-related cytokines, have recently been identified as a critical pathway for the survival of cardiac myocytes (Podewski EK *et al.*). Moreover, an impaired activation of the IL-6 pathway has been implicated in the pathogenesis of DCM (Podewski EK *et al.*). Bcl-2 family member proteins, which may be pro-apoptotic or antiapoptotic, regulate the apoptotic process (Kroemer G *et al.* 1997). Alterations in the expression of the pro-apoptotic Bax and anti-apoptotic Bcl-2 proteins have been described in patients with ischemic heart disease, DCM and heart failure (Akyurek O *et al.*). Furthermore, the transgenic over-expression of Bcl-2 has been shown to be cardioprotective by reducing the rate of cardiomyocyte apoptosis induced by ischemia-reperfusion injury (Chen Z *et al.*).

6.2. Aims

Given the fact that cytokines are produced simultaneously during the course of heart diseases, the overall effect might be due to the complex interactions between pro-apoptotic and anti-apoptotic cytokines, and also soluble cytokine receptors. Multistep control is involved, which might be different in various types of heart failure (Diwan A *et al.*). The pathogenetic mechanisms of DCM and HCM are not the same, and the exact roles of cytokines in these disorders are not yet fully understood.

The aim of our study was therefore to compare the contributions of TNF-α and IL-6 to the pathogenesis of DCM and HCM, and to explore a potential connection between the cytokine pattern and the pro-apoptotic or anti-apoptotic markers in these two different heart diseases.

6.3. Materials and Methods

Patients

The details relating to the severity of the heart failure of the patients in the different etiological groups are listed in Table 2.

	Age (years)	NYHA	LVEF (%)	LVEDD (mm)
DCM (n=31)	50	4	29	69
HCM (n=19)	51	1	72	46

NYHA= New York Heart Association; LVEF= left ventricular ejection fraction; LVEDD= left ventricular end diastolic diameter

The diagnosis of DCM was based on clinical, serological, echocardiographic and histological data. Patients were examined by right and left heart catheterization. All had an enlarged left ventricle [LVEDD ranging from 58 to 87 mm (median 69 mm)], with a LVEF reduction of from 14 to 59% (median 29%), according to the World Health Organization criteria. Cases of left ventricular dilatation due to coronary heart diseases, valvular dysfunction, hypertension, endocrine disorders or alcohol abuse were excluded. The diagnosis for HCM was based on clinical, electrocardiographic and echocardiographic data. The diagnostic criteria for HCM were defined by a maximum wall thickness > 13 mm on echocardiography, or major abnormalities on ECG (abnormal Q waves or marked T wave inversion). The healthy control population comprised 20 blood donors attending the South Hungarian Regional Blood Bank of the National Transfusion Service. All patients gave their written consent before participating. The institutional Ethical Committee approved the study.

Enzyme-linked immunosorbent assays (ELISAs) for* TNF-α, *IL-6, the soluble form of the IL-6 receptor (sIL-6R), and the soluble form of Fas (sFas).

Blood samples were taken from the 20 healthy controls (median age: 50 years, range: 22 to 67 years; 14 men (70%) and 6 women (30%)), 31 DCM patients (median age: 50 years, range: 24 to 75 years; 24 men (77.4%) and 7 women (22.6%)) and 19 HCM patients (median age: 51 years, range 20 to 77 years; 12 men (63.2%) and 7 women (36.8%)) into tubes containing $EDTANa_2$. The blood samples were centrifuged for 10 min at 5000 rpm and the plasma was separated. The plasma concentrations of TNF-α, IL-6, sIL-6R and sFas were measured with ELISA kits (BioSource SA, Nivelles, Belgium, for TNF-α; R & D Systems, Inc. Minneapolis, MN, USA, for IL-6, sIL-6R and sFas), according to the manufacturers' directions. Supplied standards were used to generate the standard curves. The absorbance at 450 nm was converted to international units per millilitre (IU/ml) for TNF-α, to picograms per millilitre (pg/ml) for IL-6, and to nanograms per millilitre (ng/ml) for sIL-6R and for sFas. The detection limit of the assay was 1 IU/ml for TNF-α, 0.2 pg/ml for IL-6, 1.5 pg/ml for sIL-6R, and 20 pg/ml for sFas.

Western blot assays

Endomyocardial biopsies were obtained from DCM patients, myocardial tissue specimens from the hypertrophied septum of patients undergoing septal myectomy for severely symptomatic HCM, and control myocardium from victims of sudden accidental death (within 6 h following death). Total protein was solubilized after ultrasonication of the myocardial tissue specimens, with subsequent processing in Laemmli's sample buffer. The individual protein samples were resolved by SDS-PAGE and electrotransferred onto

nitrocellulose filters (Amersham, Buckinghamshire, UK). Preblocked blots were reacted with specific primary antibodies to TNF-α (R & D), IL-6 (R & D), Bax (Santa Cruz Biotechnology Inc., Cambridge, MA, USA) or Bcl-2 (Serotec Ltd, Oxford, UK) for 4 h in PBS containing 0.05% (v/v) Tween 20, 1% (w/v) dried non-fat milk (Difco Laboratories, Detroit, MI, USA), and 1% (w/v) BSA (fraction V; Sigma Chemical Co., St. Louis, MO, USA). Blots were then incubated for 2 h with anti-mouse secondary antibody coupled to peroxidase (Amersham). Filters were washed five times in PBS-Tween for 5 min between each step, and were developed with a chemiluminescence detection system (Amersham).

Statistical analysis

Plasma TNF-α, IL-6, sIL-6R and sFas concentrations are expressed as medians; box plots show the medians, 25th and 75th percentiles, and ranges. Statistical analysis was performed by using the Mann-Whitney test. Differences were considered to be statistically significant at $p < 0.05$.

6.4. Results

Plasma levels of TNF-α, sFas, IL-6 and sIL-6R in patients with DCM or HCM

Plasma levels of TNF-α, sFas, IL-6 and sIL-6R were measured by ELISA in the 31 DCM patients, the 19 HCM patients and the 20 control subjects. A significantly higher TNF-α concentration was observed in the DCM patients as compared with the HCM patients and the controls (the median TNF-α concentration in the DCM patients was 75 (range, 22.5 to 133.5) versus 2.0 (range, 0 to 7.5) IU/ml in the HCM patients, $p < 0.0001$, and 1.5 (range, 0 to 6) IU/ml in the control subjects, $p < 0.0001$) (Figure 17). No significant difference in median TNF-α level was found between the HCM patients and the controls.

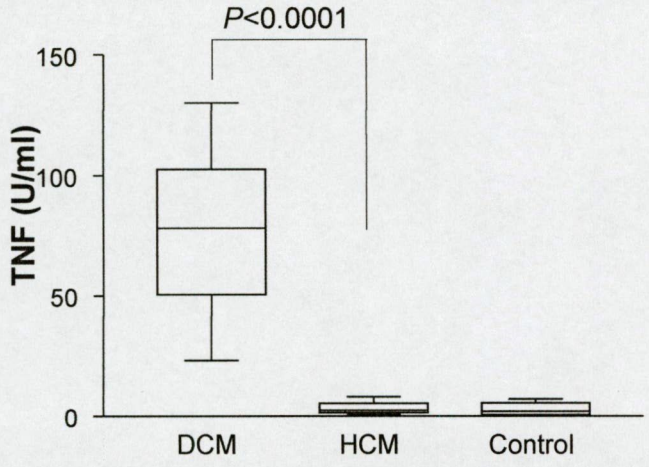

Figure 17. Plasma levels of TNF-α in patients with DCM or HCM. Plasma samples obtained from 20 healthy blood donors, 31 patients with DCM and 19 patients with HCM were assayed for TNF-α by means of ELISA. The box plots show the medians, 25th and 75th percentiles, and ranges

The DCM patients exhibited a significantly higher sFas concentration in comparison with the HCM patients and the controls (the median sFas concentration in the DCM patients was 59.5 (range: 6.0 to 141.3) versus 2.8 (range, 0 to 8.0) ng/ml in the HCM patients, $p < 0.0001$, and 4.0 (range: 0 to 5.8) ng/ml in the control subjects, $p < 0.0001$) (Figure 18). The median plasma level of sFas in the HCM patients was comparable to that measured in the controls.

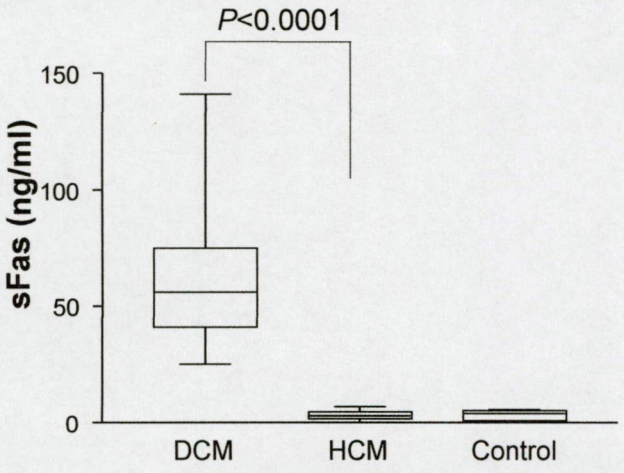

Figure 18. Plasma levels of sFas in patients with DCM or HCM. Plasma samples obtained from 20 healthy blood donors, 31 patients with DCM and 19 patients with HCM were assayed for sFas by means of ELISA. The box plots show the medians, 25th and 75th percentiles, and ranges.

Both the DCM and the HCM patient groups displayed significantly higher plasma levels of IL-6 as compared with the controls (the median IL-6 concentration in the DCM patients was 195 (range: 10 to 545) pg/ml and in the HCM patients was 250 (range: 90 to 750) pg/ml versus 7.5 (range: 5 to 10) pg/ml in the control subjects, $p < 0.0001$, for both) (Figure 19).

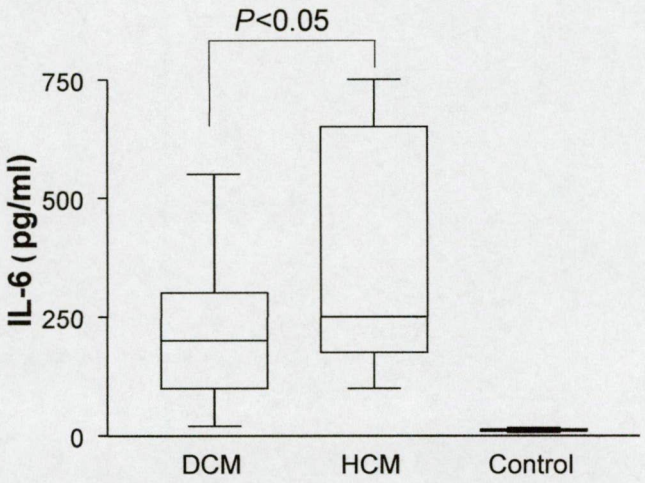

Figure 19. Plasma levels of IL-6 in patients with DCM or HCM. Plasma samples obtained from 20 healthy blood donors, 31 patients with DCM and 19 patients with HCM were assayed for IL-6 by means of ELISA. The box plots show the medians, 25th and 75th percentiles, and ranges.

Furthermore, the assay revealed a significantly higher median IL-6 concentration in the HCM patients than in the DCM patients ($p < 0.05$). In both the DCM and the HCM patient groups, there were highly increased plasma concentrations of sIL-6R as compared with the controls (the median sIL-6R concentration in the DCM patients was 22.2 (range: 11.0 to 28.0) ng/ml and in the HCM patients was 30.3 (range, 17.3 to 40.0) ng/ml versus 16.2 (range: 6.8 to 17.9) ng/ml in the control subjects, $p < 0.01$ and $p < 0.001$, respectively) (Figure 20). Moreover, the assay revealed a significantly higher median sIL-6R concentration in the HCM patients than in the DCM patients ($p < 0.001$).

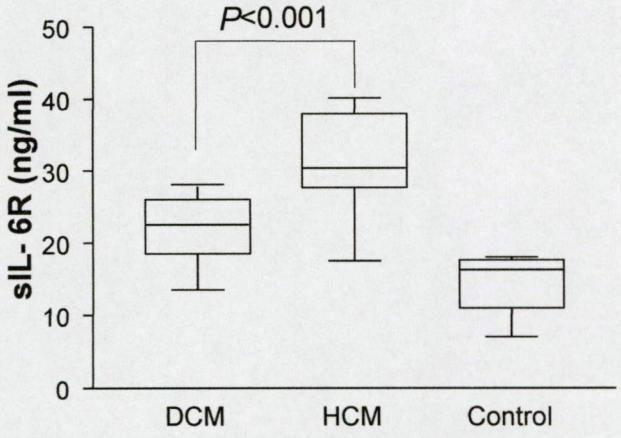

Figure 20. Plasma levels of sIL-6R in patients with DCM or HCM. Plasma samples obtained from 20 healthy blood donors, 31 patients with DCM and 19 patients with HCM were assayed for sIL-6R by means of ELISA. The box plots show the medians, 25th and 75th percentiles, and ranges.

Thus, in the DCM patients, the levels of TNF-α, sFas, IL-6 and sIL-6R were elevated, while only the IL-6 and sIL-6R levels were elevated in the HCM patients as compared with the healthy individuals. Moreover, there were significant differences between the two patient groups: the plasma TNF-α and sFas levels were higher in the DCM patients (Figures 17 and 18, $p < 0.0001$ for both), while the plasma IL-6 and sIL-6R concentrations were higher in the HCM patients (Figures 19 and 20, $p < 0.05$ and $p < 0.001$, respectively)

Expressions of TNF-α, IL-6, Bcl-2 and Bax proteins in the myocardium of patients with DCM or HCM

The steady-state levels of TNF-α, IL-6 and Bc1-2 proteins in myocardium specimens from patients with DCM or HCM were determined by means of Western blot analysis. Figure 21 presents representative results for 2 DCM and 2 HCM samples.

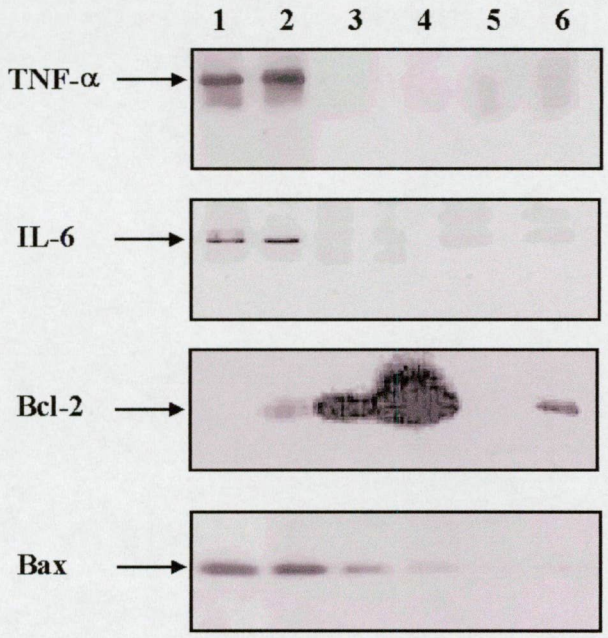

Figure 21. Expressions of TNF-α, IL-6, Bcl-2 and Bax proteins in myocardial tissue from patients with DCM or HCM. Total protein was isolated from myocardial tissue samples from control subjects (lanes 5 and 6) and patients with DCM (lanes 1 and 2) or HCM (lanes 3 and 4). Samples were resolved on 12% SDS–PAGE and transferred to nitrocellulose filters. The steady-state levels of TNF-α, IL-6, Bcl-2 and Bax proteins were analyzed by Western blot assay, using the corresponding antibodies.

TNF-α could not be detected in the heart lysates of the control subjects and the HCM patients (Figure 21, lanes 5, 6 and 3, 4, respectively). However, the DCM samples contained elevated levels of this cytokine (Figure 21, lanes 1 and 2).

Likewise, IL-6 was not detected in the myocardium of the healthy individuals and the HCM patients (Figure 21, lanes 5, 6 and 3, 4, respectively). In contrast, the DCM specimens revealed highly elevated IL-6 levels (Figure 21, lanes 1 and 2).

The control and DCM heart lysates exhibited low and variable Bcl-2 expression levels (Figure 21, lanes 5, 6 and 1, 2, respectively), whereas the levels in the HCM heart lysates were highly elevated (Figure 21, lanes 3 and 4).

The control and HCM heart lysates exhibited low levels of expression of Bax (Figure 21, lanes 5, 6 and 3, 4, respectively), whereas those in the DCM heart lysates were considerably higher (Figure 21, lanes 1 and 2).

Together, these results indicate that the myocardial tissues from the DCM patients express increased levels of TNF-α, IL-6 and Bax, while the HCM heart lysates exhibit only elevated levels of Bcl-2.

6.5. Discussion

Different plasma levels of TNF-α, sFas, IL-6 and sIL-6R in DCM and HCM patients

The cardiocytotoxicity of TNF-α observed in a variety of clinical conditions is not only due to the negative inotropic effects of TNF-α, but may also be complicated by TNF-α-induced cell death via apoptosis (Song W *et al.*, Feldman AM *et al.*). The level of plasma TNF-α, an inducer of apoptosis, was significantly higher in the DCM patients. In these patients, an elevated plasma concentration of sFas was also observed. Fas is an apoptosis-signaling surface receptor belonging in the TNF receptor family, which is known to trigger programmed cell death (Yamaoka M *et al.*). FasL, the physiological agonist of Fas, is also a transmembrane protein, with homology to the TNF family in its extracellular domain. Fas and FasL have been observed as soluble molecules, in addition to their membrane-associated forms, suggesting additional complexity as concerns regulation of the apoptotic mechanism. The level of the sFas, similarly an apoptosis-signaling receptor, has been found to be elevated in patients with DCM (Sliwa K *et al.*). In accordance with the findings of that study, we observed a markedly increased plasma sFas concentration in our patients with DCM. Cells might cleave and release or synthesize sFas as a consequence of apoptosis (Nishigaki K *et al.*), but the pathophysiological significance of sFas in DCM remains unclear. Further, we compared the plasma TNF-α and Fas levels in DCM not only with those in healthy subjects, but also with those in 19 HCM patients, and found them to be significantly higher in the DCM patients than in either the healthy blood donors or the HCM patients.

In contrast, no elevation of plasma TNF-α or sFas was observed in the HCM patients, whereas significantly higher IL-6 and sIL-6R concentrations were measured than in either the controls or the DCM patients. IL-6 and sIL-6R together may contribute to cardiac hypertrophy (Woller KC *et al.* 2001, Hirota H *et al.*). A complex of IL-6 and IL-6R (either in a membrane-anchored form or in an extracellular soluble form) associates with gp130 to induce its homodimerization. The gp130-mediated signals have a physiological role in cardiomyocyte regulation and, when overstimulated, lead to cardiac hypertrophy (Wollert KC *et al.* 2001). The IL-6/sIL-6R complex can mimic the actions of other members of this cytokine family, including leukemia inhibitory factor, ciliary neurotrophic factor, and cardiotrophin-1. Cardiotrophin-1 induces myocyte hypertrophy (Pennica D *et al.*). A

hypertrophic response of cultured heart muscle cells to the IL-6/sIL-6R complex has been observed (Hirota H *et al.*). As the highest level of sIL-6R was observed in HCM patients, it is tempting to speculate that high sIL-6R and IL-6R levels may contribute to cardiac hypertrophy.

Different expressions of TNF-α, IL-6, Bcl-2 and Bax in tissue samples from DCM and HCM patients

Apoptosis is critically dependent upon the expression of the Bcl-2 family member proteins, including Bcl-2 itself, which protects cells from apoptotic triggers, and Bax, which promotes apoptosis (Reed JC 1994). It is noteworthy that only a low basal Bcl-2 expression was demonstrated in cardiac tissues from our DCM patients, while the expression of Bax was upregulated. Previous studies have demonstrated that the biological consequence (*i.e.* induction or inhibition of apoptosis) of the joint effect of the pro-apoptotic and anti-apoptotic Bcl-2 family members depends on their stochiometric ratio (Oltvai Z *et al.*). The increased ratio Bax/Bcl-2 observed in the myocardial tissues of our DCM patients may reflect the higher susceptibility of the cells to apoptotic stimuli and we therefore presume that the cardiac muscle in these patients is not well protected from apoptotic triggers. At the same time, a considerable local TNF-α expression was detected in these cardiac biopsy specimens. Since TNF-α may be produced by the cardiac cells themselves (Torre-Amione *et al.*), it is possible that the local concentration of TNF-α is higher in the cardiac tissue, and can trigger apoptosis. These findings suggest that the elevated TNF-α levels seen in DCM patients, together with local TNF-α production, may contribute to TNF-α-induced cardiac cell death. IL-6 produced locally might be a consequence of the activation of the cytokine cascade, and the effect of IL-6 on muscle wasting might further influence the pathomechanism in DCM (Sharma R *et al.*). In the cardiac tissue samples from the HCM patients, a strong Bcl-2 expression was observed without the presence of TNF-α, IL-6 and Bax. These data indicate an imbalance between pro-apoptotic and anti-apoptotic signals.

In conclusion, these results suggest that the relatively high levels of TNF-α and sFas in DCM patients, together with the local TNF-α and Bax expressions, are signs of apoptotic processes. In contrast, in HCM patients, the high IL-6 level, together with high sIL-6R concentrations, can influence the hypertrophic processes through gp130 signaling. Moreover, there is a sharp difference between the local Bcl-2 expressions, and a considerable upregulation of the anti-apoptotic Bcl-2 is proposed in HCM. These findings have a bearing on the pathogenesis of the cardiomyopathies, since apoptosis may account for certain dysfunctions observed in DCM, while IL-6 may elicit the hypertrophy characteristic of HCM.

It is obvious that cytokines with apoptotic or hypertrophic effects can not be the sole causative agents of cardiac diseases, but they can influence the magnitude and extent of irreversible tissue damage. The basic pathological condition and the histological impairments in the cardiac tissue, together with the circulatory problems, may determine the cytokine pattern and *vice versa*. An understanding of the injury process involving cytokines may result in a better mode of intervention and thus in a reduction in cardiac damage.

7. GENERAL CONCLUSIONS, NEW FINDINGS

This thesis summarizes some of the most important results attained at the Department of Cardiac Surgery at the Medical School of the University of Szeged. One of the great achievements of modern surgery is teamwork, and the encouraging spirit and commitment of my coworkers and collaborators created an environment that has given me the opportunity to employ a number of traditional and new procedures and apply them in daily practice. This has led to the development and refinement of special cardiac surgery techniques too. Moreover, the operating theater may serve as a good interface between clinicians and basic scientists to provide an optimal environment for innovations and collaboration.

- Despite the lack of adequate funding and support, innovative technical approaches can be safely performed with no increased risk as compared with conventional cardiac surgery.
- The OPCAB technique is safe and provides good results for the patients with coronary heart disease.
- In Hungary, cryopreservation of human allografts for cardiac surgery was first implemented by our Department. The homograft bank provides a safe, cost-effective and continuous supply of biocompatible valvular substitutes and conduit materials for a wide range of cardiac interventions. The human allografts can be extensively used in different types of valvular and congenital diseases with good clinical results. Human saphenous vein allograft is a good alternative biomaterial for the performance of a modified Blalock-Taussig shunt.
- Cytokines and apoptotic processes have a role in the development and progression of cardiomyopathies. The plasma levels of TNF-α and sFas were higher in the DCM patients, while higher plasma IL-6 and sIL-6R concentrations were measured in HCM patients. DCM patients express increased levels of myocardial TNF-α, IL-6 and Bax, while the HCM myocardium exhibits only elevated Bcl-2. The results indicate an activation of the pro-apoptotic TNF and Fas pathways in DCM patients and an anti-apoptotic shift in HCM patients.

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